University of Alberta

β-Adrenergic Receptor Activation Primes Metaplasticity in Area CA1 of the Hippocampus

by

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I dedicate this thesis to my family for having always believed in me, to my love, Vanessa, and our son for all the support, care and patience during this journey.

Abstract

Neurons have the ability to change the strength of their synaptic connections in a process called synaptic plasticity. Neuromodulators such as noradrenaline can regulate synaptic plasticity in the hippocampus, which is critical for making enduring memories. Endogenous noradrenaline acts through hippocampal beta-adrenergic receptors (β-ARs) to alter synaptic efficacy. These receptors can regulate long-term potentiation (LTP), an enhancement in synaptic transmission believed to be a cellular mechanism for memory formation in the brain. I show here that isoproterenol (ISO), a β-AR agonist, when briefly applied 1 h before electrical stimulation, can facilitate late-LTP (L-LTP) in area CA1 of the hippocampus. This ability to modify the threshold for future LTP induction is called 'metaplasticity', and it can assist in keeping synapses in a state of maximal readiness for excitatory input. In this thesis, I identify novel mechanisms underlying β-AR-dependent metaplasticity. These include protein synthesis and activation of cAMP-dependent protein kinase.

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I want to thank all my family for their belief and trust in me. They believed in me, even when I did not believe in myself. My most truthful thanks to my love, Vanessa Contreras, for sharing good and bad moments. Her presence beside me gave me the harmony and happiness to achieve my goals. When I needed her the most, she was always there for me. And to our son, Giovanni, the most special gift in our lives.

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List of Abbreviations

ACSF - Artificial Cerebrospinal Fluid
ACT-D – Actinomycin D
AMPAR - alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANS - Anisomycin
APV - DL-2-Amino-5-phosphonopentanoic acid
β-ARs - beta-Adrenergic Receptors
cAMP - Cyclic Adenosine Monophosphate
CA1 - Cornu Ammonis 1
DMSO – Dimethyl Sufoxide
DPT - Depotentiation
EC - Entorhinal Cortex
eEF2 - Eukaryotic Translation Elongation Factor 2
eIF4E - Eukaryotic Initiation Factor 4E
eIF4F - Eukaryotic Initiation Factor 4F
eIF4G - Eukaryotic Initiation Factor 4G
ERK - Extracellular-Signal Regulated Kinase
fEPSP - Field Excitatory Post Synaptic Potential
HFS - High Frequency Stimulation
ISO - Isoproterenol
LC - Locus Ceruleus
LFS - Low Frequency Stimulation
LTD - Long-Term Depression
LTP - Long-Term Potentiation
LTM - Long-Term Memory
MnK1 - MAP Kinase Interacting Kinase 1
mTOR - Mammalian Target of Rapamycin
MEK - Mitogen-Activated Protein Kinase Kinase
NMDAR - N-methyl-D-aspartate Receptor
NA - Noradrenaline
PABP - Poly (A) Binding Protein
PKA - cAMP-Dependent Protein Kinase
RSK - Ribosomal S6 Kinase
STM - Short-Term Memory
S6K - RPS6-p70-Protein Kinase
TBS - Theta Burst Stimulation
4E-BP - Eukaryotic Initiation Factor 4E Binding Protein

Chapter 1 Introduction

"We remember what we understand; we understand only what we pay attention to; we pay attention to what we want." - Edward Bolles

1.1. Memory

Memory is extremely important in everyday life. The ability to recall a name, a fact or a detail about someone or something is critical for everyday survival in human society. On the other hand, memory dysfunction or amnesia can impair the ability to achieve personal goals and therefore can have devastating consequences for individuals and society. Several neurological disorders, such as Alzheimer's disease, epilepsy, stroke, and Huntington disease, can impair memory formation and synaptic plasticity. The latter is widely believed to underlie the formation and storage of some types of memory in the mammalian brain (Martin et al., 2000).

1.2. Synaptic Plasticity

Synaptic plasticity is the activity-dependent modification of synaptic strength. For the last few decades, researchers have been trying to elucidate the mechanisms of memory, and the main target of these studies has been synaptic plasticity, which has been correlated with learning and the formation of new memories in the brain (for a review, see Martin et al., 2000).

Postsynaptic responses to the release of neurotransmitter are not necessarily the same over time; in fact, these responses change depending upon the intensity and duration of the electrical or chemical stimulation applied. The ability to learn and form memories is believed to importantly involve the capacity of neurons to modify synaptic transmission, through synaptic plasticity.

One of the greatest challenges in neuroscience is to determine *how* synaptic plasticity and learning and memory are linked. Such insights are essential in order to understand the nature of diseases that affect memory systems such as Alzheimer's disease and dementias.

1.3. Long-Term Potentiation

Long-term potentiation (LTP) and long-term depression (LTD) are both forms of synaptic plasticity that are widely considered to be possible synaptic mechanisms underlying certain types of learning and memory. LTP is a lasting increase in synaptic strength and it is usually induced by one or more trains of high frequency stimulation. LTP has long been believed to represent a mechanism involved in information storage in the brain (Bear and Malenka, 1994). The focus of my thesis will be on LTP; for a review of LTD, see Bear and Abraham (1996).

The high-frequency stimulation applied to induce LTP is similar to the kind of stimulation neurons receive during intense activity (e.g. learning tasks), making LTP a prime candidate mechanism for some types of learning and memory. However, LTP is

not a single process. There is a large number of receptors, molecules and signaling pathways that can be recruited to elicit LTP.

LTP can be divided into two distinct temporal forms: early LTP (E-LTP), which is protein synthesis-independent, and late LTP (L-LTP), which requires macromolecular synthesis (Nguyen et al., 1994; Frey et al., 1996; Frey and Morris, 1997; Aakalu et al. 2001; Kandel, 2001; Sutton and Schuman, 2006). Nguyen et al. (1994) have shown that some forms of L-LTP require transcription.

The mechanisms of memory are not yet fully understood; however, the main pathways involved in this process have been elucidated. Protein kinases and phosphatases, as well as glutamatergic and neuromodulatory receptor activation and the synthesis of new proteins are all involved in memory formation (Kandel, 2001). Interestingly, many of these signalling factors are also critical for synaptic plasticity (Kandel, 2001).

1.4. The Hippocampus

The most studied brain region in modern neuroscience research is the hippocampus. Located in the medial temporal lobe, the hippocampus has an important role in the formation of spatial and declarative memories and it is critical for converting immediate or short-term memories (STM) into long-term memories (LTM) (Scoville and Milner, 1957; Zola-Morgan et al., 1986). In patients suffering from Alzheimer's disease, for example, hippocampal degeneration is highly correlated with severe memory deficits.

The hippocampus forms a network with input from the Entorhinal Cortex (EC) that connects to the dentate gyrus and area CA3 pyramidal neurons via the perforant path. CA3 neurons connect to CA1 pyramidal cells via the Schaffer collateral pathway. CA1 neurons in turn send the hippocampal output back to the EC.

Synaptic plasticity in the hippocampus can be critically modified by neuromodulators. These neuromodulators are able to alter neuronal responses and directly impact numerous brain functions such as sleep, attention, perception, learning and memory. The mechanisms by which these neuromodulators affect neuronal responses are still being investigated.

1.5. Noradrenergic Neuromodulatory System

One important neuromodulatory system in the mammalian brain is the noradrenergic neuromodulatory system. This system has been shown to have a direct effect on synaptic plasticity and LTP. The noradrenergic projections coming from the locus ceruleus nucleus (LC) innervate numerous brain areas, such as spinal cord, amygdala, hypothalamus, hippocampus, and other brain structures. Noradrenaline (NA) modulates target cells by binding to and activating alpha- and beta-noradrenergic receptors (for a review, see Gelinas and Nguyen, 2007). Depending on which types of receptors are being expressed, excitatory or inhibitory effects on synaptic strength may be observed (Gelinas and Nguyen, 2007).

Katsuki et al. (1997) have shown that activation of adrenergic receptors by 10μM NA does not affect the magnitude of LTP caused by theta burst stimulation (TBS).

Possible changes in the threshold could not be detected by this protocol, probably because this electrical stimulation protocol by itself already leads to a robust LTP. Nevertheless, a clear effect on the induction of LTD was observed when NA was co-applied with low frequency stimulation. LTD induction was inhibited, showing a shift in the threshold for LTD induction.

These results, however, do not mean that NA application only affects LTD. Co-application of NA (10μM) paired with TBS is not the best protocol to show the effects of activation of noradrenergic receptors on LTP because the TBS alone is able to induce long lasting effects on LTP. The ideal protocol would involve a noradrenergic receptor agonist paired with a subthreshold stimulus. This protocol would give us a better idea of how noradrenergic receptors modulate LTP, because it isolates a specific receptor subtype for further analysis of signalling requirements.

Another study has shown that NA infused into the hippocampus in awake rats immediately after training caused robust memory retention of an inhibitory avoidance task 24h after infusion. However, this retention was not seen 1.5h after infusion. This study suggests that NA may have a more important role in long-term than short-term memory (Izquierdo et al., 1998).

Walling and Harley (2004), by using glutamatergic activation of LC in freely moving rats, have investigated whether or not activation of LC would affect short-term memory (STM) and/or long-term memory (LTM). They observed a potentiation in the

field EPSP (fEPSP) 24h after activation of the LC. This same potentiation was not observed 3h post LC activation, suggesting that the release of NA affects long-term memory, but not short-term memory. More importantly, this increase in the fEPSPs was blocked by propranolol (a β-adrenergic antagonist), as well as by anisomycin (a protein synthesis inhibitor).

1.6. Role of β-Adrenergic Receptor Activation in Synaptic Plasticity and Translation

Activation of beta-adrenergic receptors (β-ARs) has been shown to modulate synaptic plasticity by increasing synaptic strength in the hippocampus (Thomas et al., 1996; Katsuki et al., 1997; Winder et al., 1999; Gelinas and Nguyen, 2005). This enhancement in synaptic strength is believed to modulate storage of information in the brain (Bliss and Collingridge, 1993; Martin et al., 2000).

β-ARs have been shown to mediate the effects of NA in LTP induction. Timolol, a β-AR antagonist, impaired the enhancement of synaptic strength caused by NA. Phentolamine, an α-adrenergic receptor antagonist, failed to inhibit NA modulation of LTP (Katsuki et al., 1997). Winder et al. (1999), using targeted deletion of β1- and/or β2-ARs, showed that β1- but not β2-ARs are critical for LTP elicited by pairing isoproterenol (ISO, a specific β-AR agonist) with 5-Hz low frequency stimulation.

β-ARs couple to guanine-nucleotide-binding regulatory Gs proteins to stimulate adenylyl cyclase activity and increase intracellular cAMP (Seeds and Gilman 1971; Maguire et al. 1977, Guo and Li, 2007). A main target of cAMP signalling is activation

of cAMP-dependent protein kinase (PKA), a kinase that is required for some forms of long-lasting LTP and for consolidation of hippocampal LTM (Frey et al. 1993; Abel et al. 1997; Nguyen and Woo 2003). As such, β-AR-cAMP signalling plays an important role in regulating synaptic transmission, synaptic plasticity and memory formation.

Previous research in Peter Nguyen's lab has shown that activation of β-ARs by 1μM ISO, specific and potent β-adrenergic receptor agonist, alone does not cause persistent changes in synaptic strength (Gelinas and Nguyen, 2005). However, activation of these receptors has been shown to decrease the threshold for L-LTP induction by stimulation protocols that normally would not be able to provoke L-LTP. In the same study, Gelinas and Nguyen (2005) have shown that a subthreshold stimulation of one train of 100-Hz (1x100Hz), when paired with ISO, can elicit robust L-LTP that lasts for 6hr at Schaffer collateral - commissural CA1 synapses. This study showed that β-AR activation facilitates the induction and stabilization of L-LTP in area CA1 by engaging local protein synthesis, and that transcription was not required in this process.

When paired with 1x100Hz electrical stimulus, β-AR-dependent enhancement of L-LTP maintenance has been shown to require pathways responsible for gating the translation initiation machinery, such as extracellular signal-regulated protein kinase (ERK) and mammalian target of rapamycin (mTOR) (Gelinas et al., 2007). The kinase, mTOR, has been shown to phosphorylate the eukaryotic initiation factor 4E (eIF4E) binding protein (4E-BP), a translational repressor whose activity is blocked by phosphorylation (Beretta et al., 1996), thereby promoting formation of the eIF4F

translation initiation complex (Figure 1.1).

The formation of this complex is modulated by ERK-dependent eIF4E phosphorylation by the MAP kinase interacting kinase 1, MnK1. Klann et al. (2004) have shown that eIF4E phosphorylation occurs via eukaryotic initiation factor 4G (eIF4G), because eIF4E has no direct binding site for MnK1. Both eIF4E and MnK1 need to be bound to eIF4G in order for this indirect phosphorylation to happen. This eIF4E phosphorylation has been suggested to increase translation (Figure 1.1).

Gelinas et al. (2007) have shown that ISO + 1x100Hz electrical stimulation increased the amount of eIF4E that co-precipitated with eIF4G (i.e. eIF4F complex), compared with controls (either treatment alone). eIF4G and 4E-BP compete for the same binding site on eIF4E. The fact that ISO + 1x100Hz increased the co-precipitation of eIF4E with eIF4G means that 4E-BP is being phosphorylated. This is the how 4E-BP has its affinity for eIF4E regulated.

After further investigation, Gelinas et al. (2007) observed that the formation of eIF4F complex is blocked by rapamycin, showing that mTOR plays an important role in the formation of this complex. More specifically, they observed that ISO + 1x100Hz increased 4E-BP phosphorylation, which was inhibited by rapamycin but not by U0126 (a MEK inhibitor that effectively blocks ERK activity). These results suggest that mTOR, but not ERK, is involved in the phosphorylation of 4E-BP and the de-repression of eIF4E to bind eIF4G.

Winder et al. (1999) have shown that ERK and mTOR do not play a role in the E-LTP generated by the 1x100Hz electrical stimulation protocol alone, suggesting that the inhibition of these kinases only affects the β-adrenergic component of this LTP. Also, E-LTP generated by 1x100Hz alone does not require protein synthesis or PKA (Huang et al., 1996).

Interestingly, noradrenergic projections to the hippocampus have been suggested to robustly enhance LTP induction and memory consolidation, for instance during periods of heightened emotional arousal (Thomas et al., 1996; McGaugh,1989, 1990; Cahill et al., 1994). As such, β-ARs likely play critical roles in gating the formation of memories for emotionally-charged, significant events that need to be retained for very long periods of time.

1.7. Metaplasticity

The change of threshold for future induction of LTP or LTD, by prior 'priming' activity or experience, is called 'metaplasticity', or "plasticity of synaptic plasticity" (Abraham, 1996; Abraham and Bear, 1996). Priming activity may consist of previous electrical stimuli, pharmacological activation of receptors, or behavioral events preceded by hormonal release (for review see Abraham, 2008). Kim and Yoon (1998) have shown that psychological insult such as stress can also be considered a priming activity for metaplasticity. Stressful situations have been shown to impair, for example, spatial memory tasks, which are hippocampus-dependent forms of learning (Diamond et al., 1996). Stress has also been shown to impair LTP and facilitate LTD in vivo

(Xu et al., 1997), suggesting that LTP, but not LTD, underlies hippocampal spatial memory.

It is important to note that the priming activity must be able to produce persistent changes in synaptic efficacy and that these changes must be able to persist across time, lasting even after the priming activity is no longer present. The changes must also be capable of modifying the synaptic response to a subsequent stimulus (see Fig. 1.2). This is the essence of "metaplasticity", as originally defined by Abraham and Bear (1996).

An interesting experiment by Huang et al. (1992) has shown that weak electrical stimuli delivered before a strong tetanus can impair later LTP induction. When the NMDAR antagonist, aminophosphonopentanoate (APV), was applied during the weak stimulation, LTP was successfully induced. Also, when the LTP-inducing stimuli was increased, LTP was again induced. This effect lasted at least 30min but no more than 1h. Taken together, these results suggest that a prior weak tetanus activates NMDARs, thereby increasing the threshold for the induction of LTP by occluding its induction.

Metaplasticity has been suggested to play an important role in normal neuronal function in order to prevent synapses from entering the state of either saturated LTP, thereby protecting against excitotoxicity or epilepsy for example, or LTD (Abraham and Tate, 1997). Although reasonable progress has been made in determining the molecular mechanisms of metaplasticity, much remains to be elucidated. For example, what roles

do β-ARs play in metaplasticity? Can β-AR activation control future LTP expression? If so, what signalling pathways are required?

1.8. Hypotheses and Specific Goals

Previous research has shown that *β*-ARs mediate the plasticity-enhancing effects of NA in the hippocampus (Walling and Harley, 2004; reviewed by Gelinas and Nguyen, 2007). Also, in studies performed on in vitro hippocampal slices, Gelinas and Nguyen (2005) have shown that pairing *β*-AR activation with subthreshold synaptic stimulation enhances LTP expression in apical dendrites located in area CA1 (Gelinas and Nguyen, 2005; see also Thomas et al., 1996; Katsuki et al., 1997;). This modulation gives new impetus towards elucidating the role of these important receptors in priming metaplasticity in the hippocampus. In my experiments here, I have used pharmacologic activation of *β*-ARs well before (1- 2-hrs) subsequent LTP induction to probe the roles of these receptors in metaplasticity. This is the key distinction between the experiments presented here and those of Gelinas and Nguyen (2005).

The main objective of my thesis is to identify which cellular signalling pathways are involved in metaplasticity and to understand the mechanisms by which activation of *β*-ARs and subthreshold stimulation can produce persistent LTP long after washout of an agonist of *β*-ARs.

The potential benefits of this research include: 1) better comprehension of the metaplastic process itself, 2) improved insights on how metaplasticity could attenuate

symptoms of certain neurological disorders and perhaps 3) contribute to a possible novel therapeutic target in order to alleviate specific neurological disorders. For example, propranolol, a beta-receptor blocker, has seen limited use to treat post-traumatic stress disorder by attenuating the consolidation of memories of traumatic events (Pitman et al., 2002; Evers, 2007; Henry et al., 2007;). Manipulation of molecular pathways downstream of the beta-receptor might provide more effective ways of specifically alleviating memory disorders than manipulations of the receptor per se, which can elicit a wide range of undesirable side-effects.

Previous studies have shown that metaplasticity plays a crucial role in keeping synapses from being physiologically saturated. Ullal et al. (1998) have demonstrated that by activating glutamatergic receptors, subsequent epileptic seizures can be prevented. This is a clear example of how metaplasticity can regulate synaptic plasticity to protect against excitotoxicity.

The hypotheses to be addressed in my thesis are:

1) Prior activation of β-ARs will enhance future LTP expression in a "silent" manner, without altering basal synaptic strength. .

2) This β-AR-dependent metaplasticity requires translation and transcription.

3) The translational kinase ERK is required for metaplasticity.

4) mTOR is essential for establishment of β-AR-dependent metaplasticity.

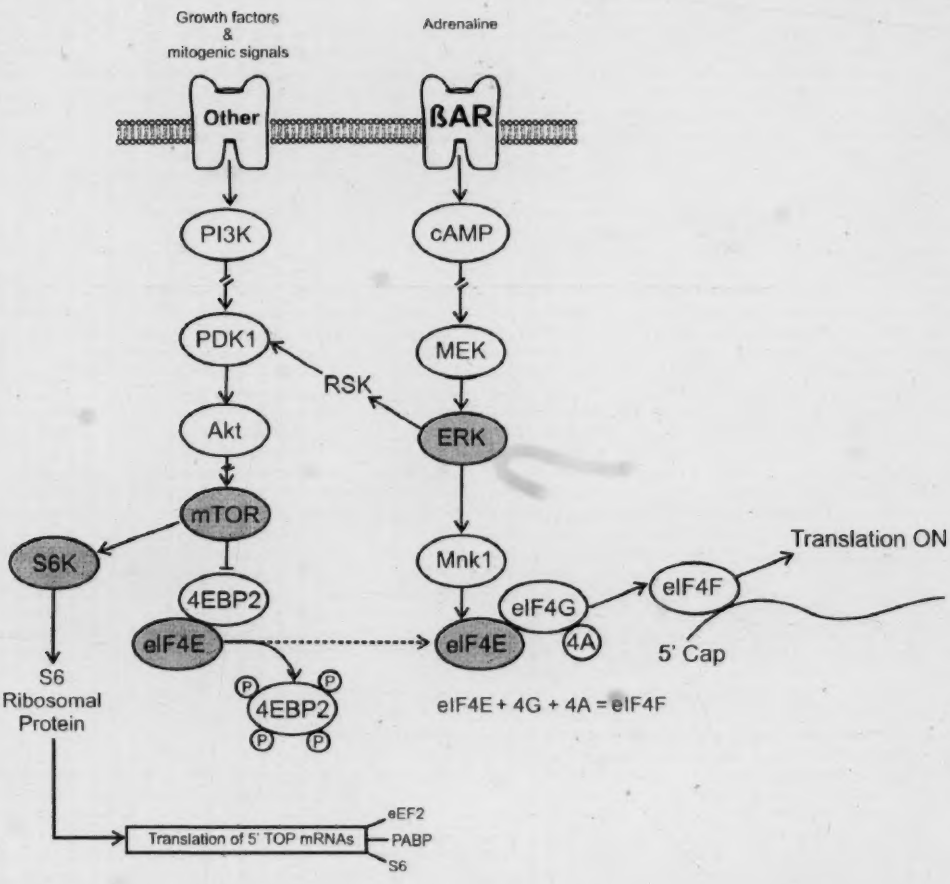

Figure1.1. Translational control by ERK and mTOR. Activation of β-ARs during synaptic stimulation promotes translation initiation through ERK and mTOR pathways. 4EBP2 acts as a translation repressor when bound to the translation initiation factor, eIF4E. Once 4EBP2 is phosphorylated by mTOR and released, eIF4E is able to form the eIF4F complex (eIF4E, 4G and 4A) and initiate translation. mTOR also activates ribosomal S6 kinase (S6K), which phosphorylates S6 to increase synthesis of translation regulatory proteins such as eEF2, PABP, and S6 itself. ERK activates Mnk1 which in turn phosphorylates eIF4E via eIF4G, increasing translation rate. ERK may cross-talk with the mTOR signalling pathway via RSK and PDK1.

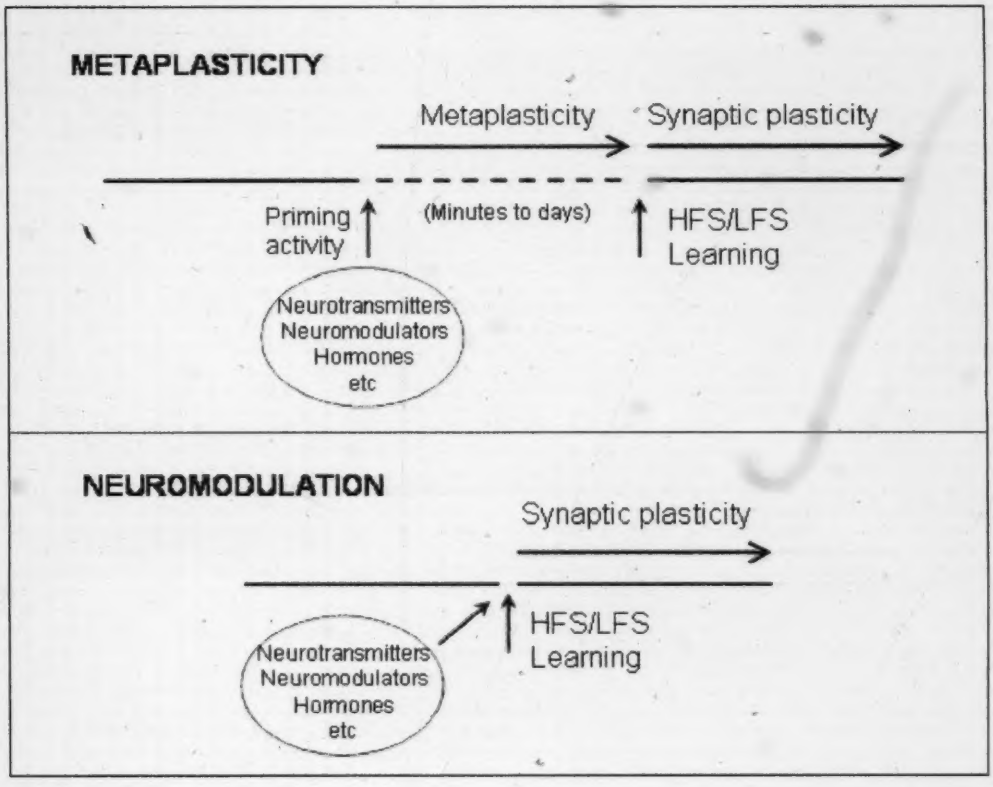

Figure 1.2. Difference between modulation of synaptic plasticity and metaplasticity. Metaplasticity refers to the situation where there is a priming activity. This activity in fact triggers changes in neural function in such a way that they persist across time even after the termination of the priming activity. This makes a distinction between metaplasticity and neuromodulation. (Abraham, 2008)

Chapter 2 Materials and Methods

2.1. *Animals.* Male C57BL/6 mice, 8–13 weeks of age (Charles River, Montreal, Quebec, Canada) were used for all experiments. Animals were housed at the University of Alberta using guidelines approved by the Canadian Council on Animal Care.

2.2. *Electrophysiology.* After cervical dislocation and decapitation as described by Nguyen and Kandel (1997), the brain was quickly transferred into ice-cold artificial cerebrospinal fluid (ACSF) composed of the following (in mM): 124 NaCl, 4.4 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 10 glucose, aerated with 95%O_2 and 5% CO_2.

Hippocampi were then dissected from the brain and transverse hippocampal slices (400μm thickness) were cut with a manual McIlwain chopper. Slices were kept in an interface chamber at 28°C and perfused (1–2 mL/min) with carbogenated ACSF (95% O2, 5% CO2) for at least 1h before recordings.

A bipolar nickel–chromium stimulating electrode (130μm diameter) was placed in the stratum radiatum to activate the Schaffer collateral/commissural fibers, and extracellular field EPSPs (fEPSPs) were recorded with a glass microelectrode filled with ACSF (resistances, 2–3 MΩ) located in the stratum radiatum of the hippocampal area CA1.Stimulation intensity (0.08 ms pulse duration) was adjusted to evoke field

excitatory postsynaptic potentials (fEPSP) that were 40% of maximal evoked size (Woo and Nguyen, 2003) (Figure 2.1.). LTP was induced by applying one train of high-frequency stimulation (HFS; 100 Hz, 1s duration). Depotentiation (DPT) was induced by applying low-frequency stimulation (LFS) consisting of 5 Hz for 3 min.

2.3. *Drugs.* Isoproterenol (ISO), a specific β-adrenergic receptor agonist, [*R* (-)- isoproterenol (+)-bitartrate, 1 μM; Sigma, St. Louis, MO] was prepared fresh daily in distilled water at 2mM stock solution. The β-adrenergic receptor antagonist propranolol [(±)-propranolol hydrochloride, 50μM; Research Biochemicals, Natick, MA] was also prepared daily in distilled water as a 50mM concentration stock solution.

The NMDAR antagonist, APV [DL-2-Amino-5-phosphonopentanoic acid, 50μM; Sigma] was prepared in distilled water as a 50mM stock solution. An mTOR inhibitor, rapamycin (1μM; Sigma) and a MEK inhibitor, U0126 (20μM; Sigma), were prepared in dimethyl sufoxide (DMSO) in stock concentrations at 1mM and 20mM, respectively. Methanol was used to dissolve the PKA inhibitor, KT5720 (1μM KT5720 final concentration in ACSF; Sigma), as a 1mM stock solution.

Two protein synthesis inhibitors, anisomycin (40μM; Sigma) and emetine (20μM; Sigma), were prepared as concentrated stock solutions at 40mM in DMSO and 20 mM in distilled water, respectively. Both anisomycin and emetine, at lower concentrations than those used here, blocked protein synthesis by > 80% in hippocampal slices (Frey et al.,

1988).

A transcriptional inhibitor, actinomycin D (ACT-D; 25 μM bath concentration; Bioshop Canada, Burlington, Ontario, Canada), was prepared in DMSO as a 25mM concentration stock solution. At the bath concentration used here, ACT-D has been shown to block transcription by >70% in hippocampal slices (Nguyen et al., 1994). Final concentrations were obtained by diluting each drug in ACSF and then ISO was bath-applied for15 min. The diluted bath concentrations of DMSO (0.1%) and methanol (0.1%) did not affect either basal synaptic transmission or LTP (data not shown).

Slices were given HFS (1x100Hz) 1 or 2h after ISO application. Propranolol, anisomycin, emetine, ACT-D, and rapamycin were applied at 30 min before ISO application, and they were present throughout ISO application and 10 min after ISO application.

KT5720 and U0126 were applied for 20 min before ISO and remained in the bath for an additional 25 min thereafter. APV application overlapped with HFS. All drug experiments were performed under dimmed light conditions because of the photosensitivity of drugs. Drug experiments were interleaved with drug-free controls.

2.4. *Data analysis*. Measurements of the fEPSP slopes were made as an indication of synaptic strength (Johnston and Wu, 1995). Baseline fEPSP slopes were measured during the first 20 min of recording and then compared to fEPSP slopes measured 120 min after HFS for comparisons of LTP (Woo and Nguyen, 2003).

Results are presented as mean ± standard error of the mean (S.E.M.) if not indicated otherwise, with n = number of slices. Statistical analysis was assessed by using an unpaired Student's t test, to compare mean fEPSP slopes between two groups, with a significance level of $p < 0.05$.

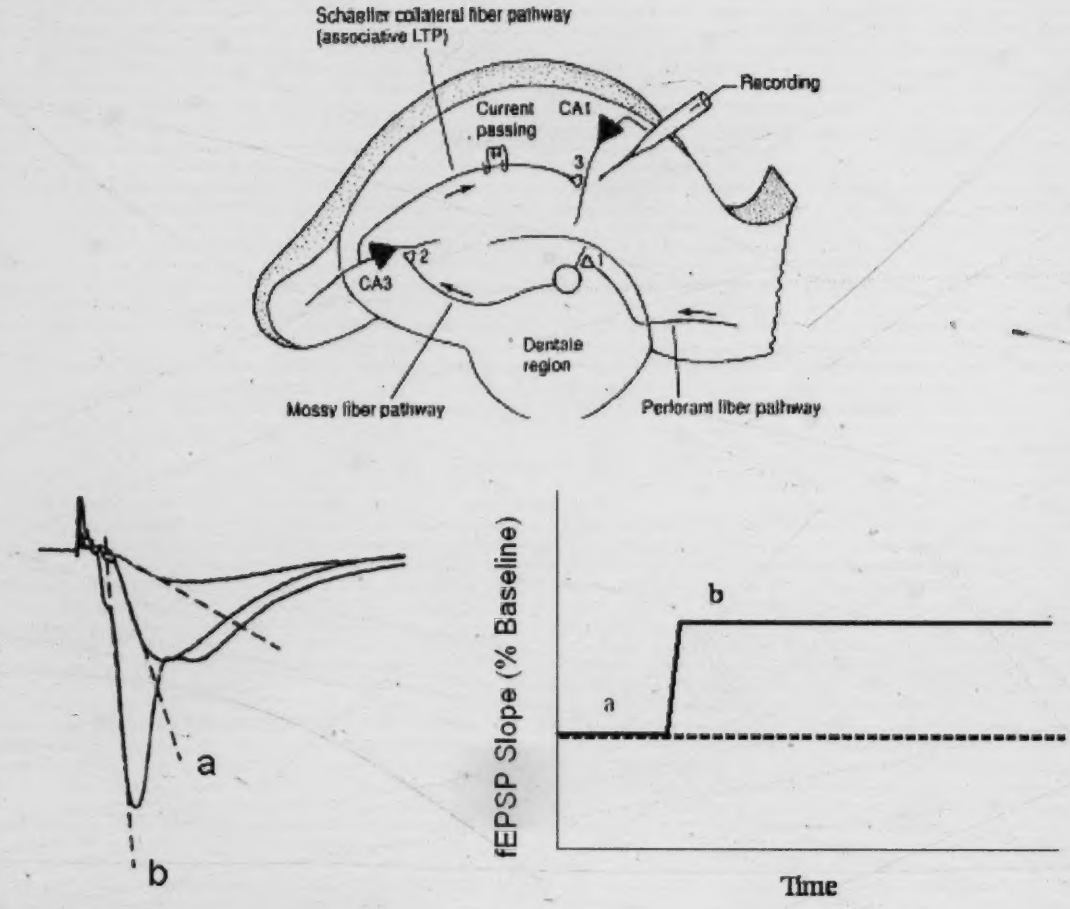

Figure 2.1. LTP in the CA1 region of hippocampal slices. Top: Diagram showing positions of stimulation and recording electrodes. Bottom: Initial slopes of field EPSPs are measured before (a) and after (b) high-frequency stimulation that induces LTP. Results are graphed as shown schematically. [Top diagram modified from "Principles of Neural Science", 4th ed., E. Kandel et al., eds.]

Chapter 3 Results

3.1. Activation of β-ARs Reduces the Threshold for Metaplasticity in Area CA1 of the Hippocampus

In the mammalian brain, neuromodulatory transmitters control memory formation and the endurance of synaptic plasticity. Noradrenaline (NA) is one important transmitter that critically modulates the longevity of both LTP and hippocampal memories by acting on β-ARs. Previous research has shown that β-AR activation can facilitate the induction of LTP by stimulation protocols which do not normally induce persistent LTP (Thomas et al., 1996; Katsuki et al., 1997, Gelinas & Nguyen, 2005). Additionally, activation of G-protein coupled receptors (such as β-ARs) can initiate intracellular signaling cascades which alter the ability of synapses to undergo plasticity, a phenomenon known as metaplasticity (Abraham, 2008). Thus, I wanted to determine if activation of β-ARs engages metaplastic processes in mouse hippocampal slices.

In order to examine the role of β-ARs in hippocampal metaplasticity, I applied isoproterenol (ISO; 1μM), a specific β-AR agonist, for 15 minutes. Following a 1h washout period, high-frequency stimulation (HFS, 1x100Hz) was applied to the Schaeffer Collateral (SC)-CA1 pathway. I observed that pairing β-adrenergic receptor activation with 1h ISO wash out, followed by HFS, generated L-LTP that lasted for >6h (**Figure 3.1**) (fEPSPs were 144 ± 6% of baseline levels 7h after HFS).

It should be noted that HFS alone elicited only a transient increase in potentiation which returned to baseline in <2h, which is considered early-LTP (E-LTP) (Huang and Kandel, 1994) (**Figure 3.2.**) (fEPSPs were 104 ± 6% of baseline levels 120 min after

HFS). Also, application of 1μM ISO alone has been shown to have no persistent effect on basal synaptic responses (Gelinas and Nguyen, 2005). When ISO was washed out for 2h, slices treated with ISO behaved the same as controls (1x100Hz alone) (**Figure 3.2.**) (fEPSPs slopes were 103 ± 5% of baseline levels 120 min after HFS).

These results suggest that activation of β-ARs initiates metaplastic processes lasting at least 1h which facilitate the maintenance of L-LTP induced by a subthreshold stimulation protocol.

3.2 This Form of Metaplasticity Requires β-Adrenergic and NMDA Receptor Activation

Noradrenaline (NA) has been shown to have a crucial role in some forms of synaptic plasticity (Gelinas and Nguyen, 2005) and in the formation and retrieval of memories (Berridge and Waterhouse, 2003). Importantly, NA alters synaptic plasticity through activation of β-ARs, and research suggests that the β1-AR subtype is crucially involved in hippocampal responses to NA (Winder et al., 1999).

When 1x100Hz HFS was delivered 1h after β-AR activation, the persistence of L-LTP was enhanced (**Figure 3.2**) (fEPSPs were potentiated to 142 ± 7% 120 min after HFS). To test the idea that this form of metaplasticity requires β-AR activation, I applied the β-AR antagonist, propranolol. My results indicate that propranolol blocked the metaplastic effects and L-LTP was not induced (**Figure 3.3.**) (fEPSPs were 98 ± 8% of baseline levels 120 min after HFS; $p<0.01$ compared to controls). Therefore, this form of metaplasticity requires β-AR activation. When propranolol was applied 40 min after ISO application, it had no effect on the induction of L-LTP (**Figure 3.3.**) (fEPSPs were

potentiated to 143 ± 3% 120 min after HFS), which suggests that only a transient activation of β-ARs is needed to engage the metaplastic processes necessary for long-term changes in synaptic efficacy.

Activation of N-methyl-D-aspartate (NMDA) receptors is a key trigger for LTP and memory formation (Collingridge et al., 1983; Morris et al., 1990; Nicoll and Malenka, 1999). The NMDA receptor is an ionotropic glutamate receptor and its activation allows the postsynaptic influx of calcium that is essential for many forms of synaptic plasticity, learning and memory. These receptors have also been shown to modulate metaplasticity, as prior activation of NMDARs can block the future induction of LTP (Collingridge et al., 2004).

To determine if NMDAR activation is required for this form of metaplasticity, I applied 2-amino-5-phosphonovaleric acid, APV (50μM), an NMDAR antagonist. My results indicate that APV completely blocks this form of metaplasticity (**Figure 3.4.**) (fEPSPs were 105 ± 3% of baseline levels 120 min after HFS; $p<0.01$ compared with controls). Taken together, these results show that activation of both β-ARs and NMDARs is necessary for the expression of this form of metaplasticity and the subsequent induction of L-LTP.

3.3. β-Adrenergic Receptor-Dependent Metaplasticity is Immune to Depotentiation

Depotentiation is an activity-dependent reversal of LTP that can take place during a brief time interval immediately after LTP induction (Staubli and Lynch, 1990; Fujii et al., 1991; Huang et al., 1999). It is known that low-frequency stimulation (LFS, 5Hz, 3 min) can induce depotentiation (O'Dell and Kandel, 1994). Woo and Nguyen (2003)

demonstrated that protein synthesis-dependent forms of LTP are immune to depotentiating stimuli. Therefore, I wanted to determine if this form of metaplasticity was also immune to depotentiation. Following induction of L-LTP, a depotentiation stimulus protocol (5Hz, 3 min; 10 min-post induction) was applied. I observed that this form of metaplasticity is immune to depotentiation, because synaptic potentials returned to the same level of increased potentiation observed when no depotentiating stimulus was applied (**Figure 3.5.**) (fEPSPs were potentiated to 149 ± 3 % 120 min after depotentiation stimuli; $p<0.01$ compared with controls). This result suggests that metaplasticity engaged by β-AR activation may be dependent upon translation. Thus, I next asked whether or not translation is required for this metaplasticity.

3.4. Translation, but not Transcription, is Required for This Form of Metaplasticity

Because LTP lasting more than 2h has been shown to be translation-dependent (Frey et al., 1988; Nguyen and Kandel, 1996; Kandel, 2001)), I hypothesized that protein synthesis is required for this form of metaplasticity of L-LTP.

To determine if protein synthesis was necessary for L-LTP, I used the translational inhibitor, anisomycin (ANS), at a concentration that inhibits >80% of protein synthesis (Frey et al., 1988). When ANS was co-applied with ISO (1h prior to HFS) L-LTP was no longer induced (**Figure 3.6.**) (fEPSPs were 101 ± 3% of baseline levels 120 min after HFS; $p<0.05$ compared with controls), suggesting that ISO primes translation to enhance future L-LTP expression. Interestingly, application of ANS after activation of β-AR had no effect on the subsequent induction of L-LTP (**Figure 3.6.**) (fEPSPs were potentiated to 151 ± 8% 120 min after HFS). These results suggest that

β-AR activation triggers signaling cascades which prime downstream translation to control expression of future L-LTP.

Additionally, transient activation of β-ARs engages cellular mechanisms which expand the temporal window for inducing translation-dependent LTP to at least 1h. It is also worth noting that results of the experiments in which ANS was applied after ISO application (during HFS) suggest that ANS was not affecting baseline transmission, because ANS applied at this later time point had no significant effect on the amplitude or duration of L-LTP.

Recent papers have questioned the validity of using ANS as a translational inhibitor, due to the possibility of negative side effects that are independent of translational inhibition (Alberini, 2008). Therefore, I also used the protein synthesis inhibitor emetine (EME) to test for translation dependence. Again, I found that when EME was applied, the metaplastic effects were blocked (**Figure 3.7.**) (fEPSPs were 100 ± 6% of baseline levels 120 min after HFS; $p<0.01$ compared to controls). Thus, similar to other forms of translation-dependent LTP (Woo et al., 2003, Gelinas and Nguyen, 2005), this form of metaplasticity requires translation.

This finding, when combined with my ANS data, suggests that L-LTP induced through β-AR-dependent metaplasticity is critically dependent upon protein synthesis. Furthermore, my results suggest that the disruption of the metaplastic effects are not due to non-specific side effects of translation blockers, as both ANS and EME, which function in distinct ways to block protein synthesis, yielded similar results.

Some forms of synaptic plasticity require transcription. L-LTP generated by HFS requires synthesis of new mRNA (Abraham et al., 1993; Nguyen et al., 1994; Frey et al.,

1996). Nevertheless, Gelinas and Nguyen (2005) have shown that L-LTP produced by pairing β-AR activation and 1x100Hz HFS is not dependent on transcription, suggesting that some neuromodulators are able to induce long-lasting effects that are independent of transcription.

To test the hypothesis that transcription is necessary for metaplasticity, I applied actinomycin-D, ACT-D, at a concentration which blocks >70% of transcription (Nguyen et al., 1994). ACT-D had no effect on either the induction or maintenance of L-LTP initiated through metaplastic processes (**Figure 3.8.**) (fEPSPs were potentiated to 148 ± 5% 120 min after HFS). These results indicate that β-AR-dependent metaplasticity is not dependent on transcription.

In summary, the synthesis of new proteins is required for the expression of this form of metaplasticity. However, these translational processes appear to involve pre-existing mRNA, as transcription was not required.

3.5. β-AR-Dependent Metaplasticity Requires PKA and ERK but not mTOR

The signaling pathways involved in β-AR-dependent metaplasticity have yet to be fully elucidated. cAMP-dependent protein kinase (PKA) can be recruited by specific stimulation protocols (Gelinas et al., 2008) and β-AR activation (Thomas et al., 1996). Therefore, I hypothesized that metaplastic processes may recruit the PKA signaling cascade to boost synaptic responses.

The PKA antagonist KT5720 was co-applied during β-AR activation. My data show that activation of PKA was necessary for metaplasticity, as KT5720 blocked the induction of L-LTP after washout of ISO and KT5720 (**Figure 3.9.**) (fEPSPs were

103 ± 8% of baseline levels 120 min after HFS; $p<0.01$ compared with ISO plus 1x100Hz). Interestingly, Gelinas et al. (2008) found that different patterns of electrical stimulation selectively recruited distinct signaling cascades after β-adrenergic activation. Their results showed that PKA is critical for LTP induction when LFS (5Hz, 3min) was applied with ISO. However, when β-AR activation was paired with HFS (1x100Hz), PKA was no longer needed (Gelinas et al., 2008). My results suggest that PKA is required for the expression of metaplasticity when stimulation is applied 1h after ISO application.

Another kinase downstream of PKA which has been strongly implicated in LTP is the extracellular signal-regulated kinase (ERK). β-ARs can recruit ERK, as well as another kinase, mammalian target of rapamycin (mTOR) (Gelinas and Nguyen 2005; Gelinas et al. 2007), both of which have been implicated in the long-term stability of LTP (Tang et al. 2002; Kelleher et al. 2004; Sweatt, 2004). In addition, MnK1, an ERK-dependent kinase, phosphorylates the eukaryotic translation initiation factor 4E (eIF4E), which ultimately results in increased rates of translation (Klann et al., 2004).

To determine if ERK was involved in metaplasticity, I applied U0126, a mitogen-activated protein kinase kinase (MEK, which phosphorylates ERK to activate it) inhibitor. Slices treated with U0126 exhibited significantly reduced levels of potentiation following stimulation (**Figure 3.10.**) (fEPSPs were 100 ± 6% of baseline levels 120 min after HFS; $p<0.01$ compared to controls). These results suggest that ERK is required for metaplasticity induced by activation of β-adrenergic receptors.

Another important component of the translational signaling pathway implicated in β-AR-dependent LTP is mTOR, which regulates protein synthesis by phosphorylating

and inactivating a repressor of mRNA translation, eukaryotic initiation factor 4E-binding protein, 4E-BP (Beretta et al., 1996). Beretta et al. (1996) have shown that mTOR is blocked by rapamycin. 4E-BP and eIF4G compete for the same binding site on eIF4E. When 4E-BP is bound to eIF4E, translation is blocked. If eIF4G is bound to eIF4E, they are able to form the eIF4F complex, which now can bind with ribosomes and initiate translation.

Tang et al. (2001) have shown that disruption of the mTOR signaling cascade attenuates L-LTP expression generated by HFS. However, my results indicate that application of rapamycin does not block this form of metaplasticity (**Figure 3.11.**) (fEPSPs were potentiated to 143 ± 6% 120 min after HFS). Therefore, mTOR is not required for L-LTP generated through metaplastic processes.

Taken together, my results suggest the β-AR-dependent metaplasticity is mediated through a PKA-ERK dependent pathway, but does not require mTOR-dependent signaling.

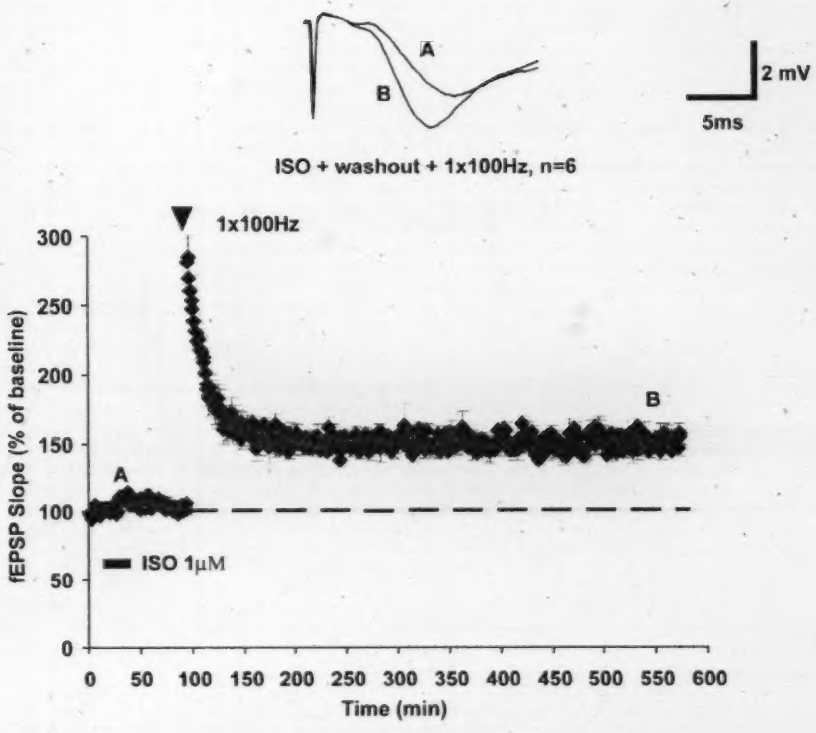

Figure 3.1. Duration of metaplasticity induced by previous activation of β-ARs. ISO was applied then washed out for 1h, followed by one train of HFS (1x100Hz) which induced L-LTP that lasted > 6h. One train of HFS alone normally induces only E-LTP that decays back to baseline within the first 2h (see Figure 3.2).

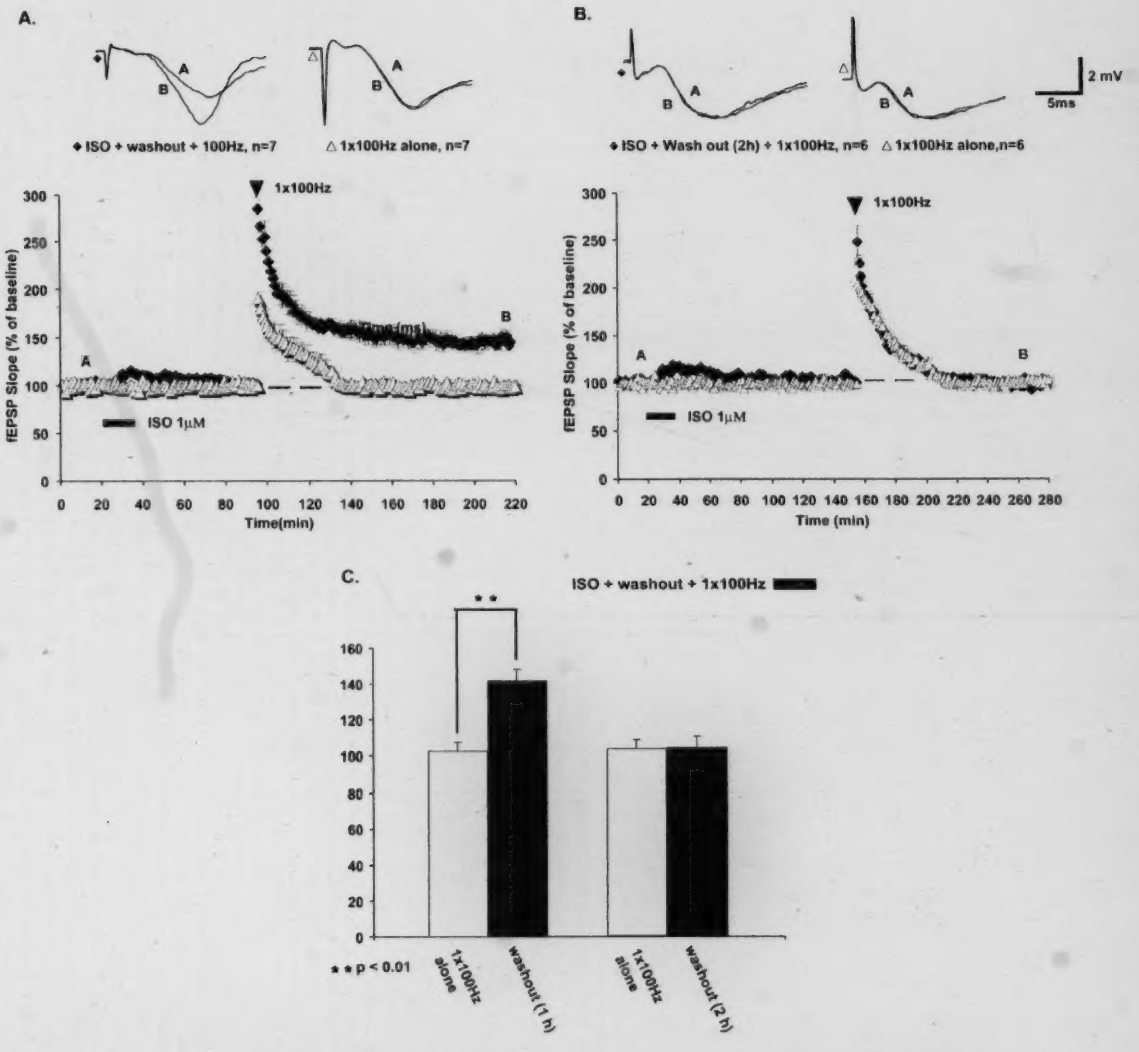

Figure 3.2. Activation of β-ARs by ISO functions as a priming stimulus, reducing the threshold for future L-LTP induction. *A*, 1x100Hz HFS alone only induces a temporary increase in synaptic efficacy that returns to baseline levels within 2 h (open triangles). *B*, However, when ISO is washed out for 2 h (black diamonds), the metaplastic effect is no longer present. *C*, Summary histogram comparing fEPSP slopes obtained 120 min after HFS. Sample traces were taken 10 min after commencement of baseline recordings and 120 min after HFS. Results in C represent means ± SEM (n=7).

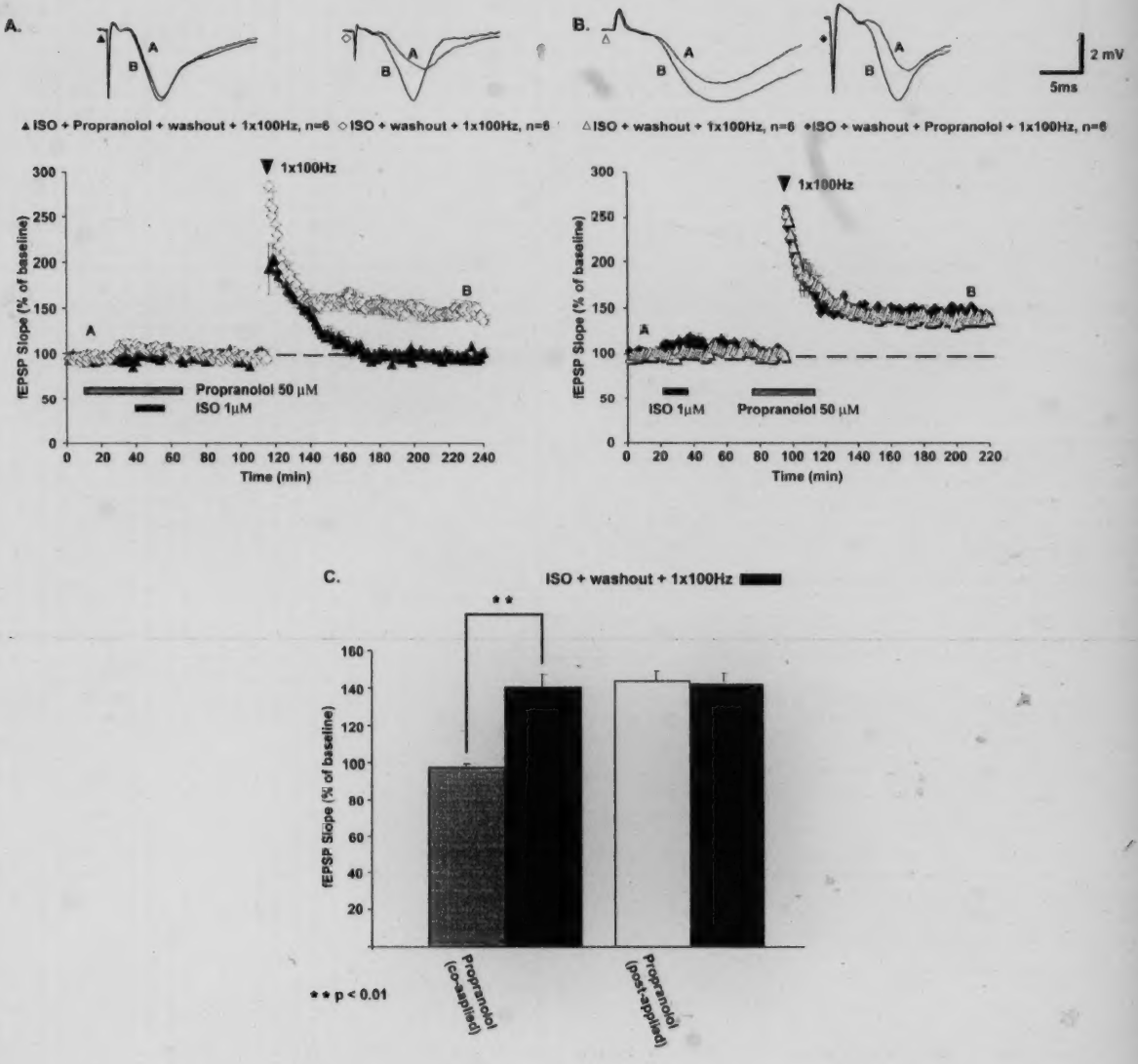

Figure 3.3. This form of metaplasticity requires β-AR activation in order to elicit L-LTP. *A*, Application of propranolol during β-AR activation inhibits metaplastic effects (triangles). *B*, The presence of propranolol during HFS does not impair L-LTP generated in this manner (black diamonds). *C*, Summary histogram comparing fEPSP slopes obtained 120 min after HFS. Sample traces were taken 10 min after commencement of baseline recordings and 120min after HFS. Results in C represent means ± SEM (n=6).

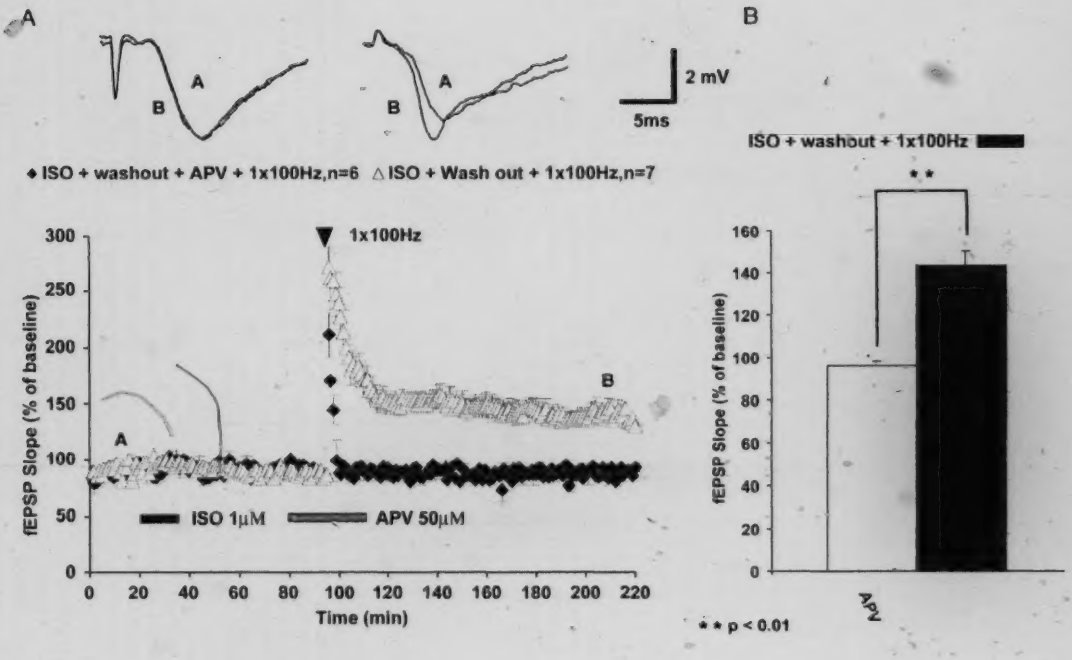

Figure 3.4. NMDAR block by APV completely impaired this form of metaplasticity. *A*, Compared to controls (open triangles), 1x100Hz HFS in the presence of APV applied after β-AR activation blocked metaplasticity (black diamonds). *B*, Summary histogram comparing fEPSP slopes 120 min after HFS (**$p<0.01$). Sample traces were taken 10 min after commencement of baseline recordings and 120 min after HFS stimulation. Results in B represent means ± SEM (n=6).

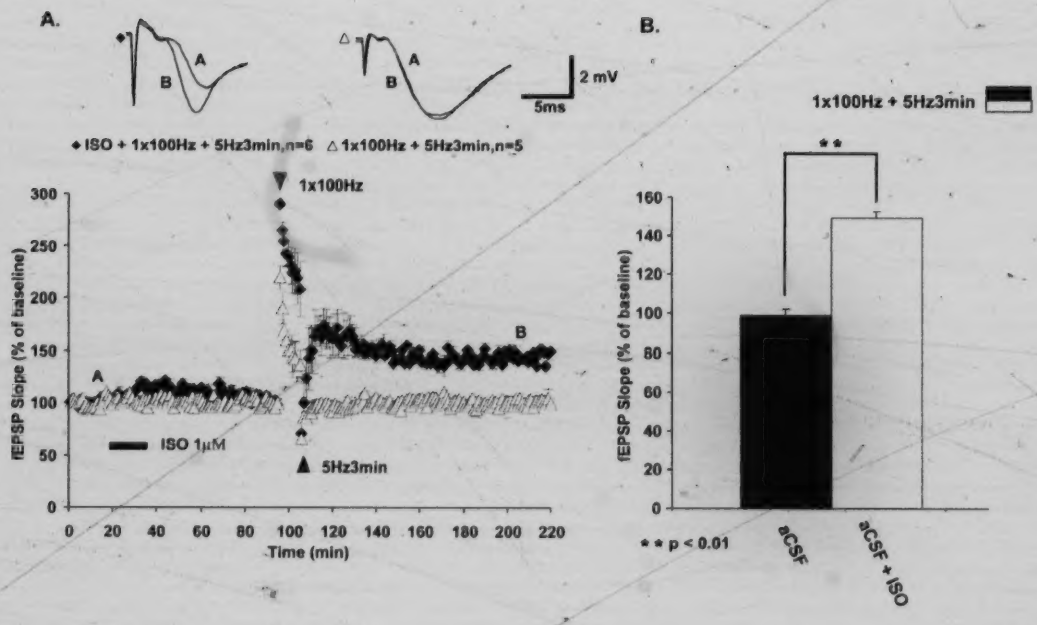

Figure 3.5. β-AR-dependent metaplasticity produced by subthreshold HFS generates L-LTP that is immune to depotentiation. *A*, Application of depotentiation stimuli (5 Hz, 3 min) 10 min after LTP induction does not affect potentiation levels when β-ARs are previously activated (black diamonds). Note gradual recovery of fEPSPs to potentiated levels. *B*, Summary histogram comparing fEPSP slopes 120 min after HFS. Sample traces were taken 10 min after commencement of baseline recordings and 120min after HFS stimulation. Results in B represent means ± SEM (n=6).

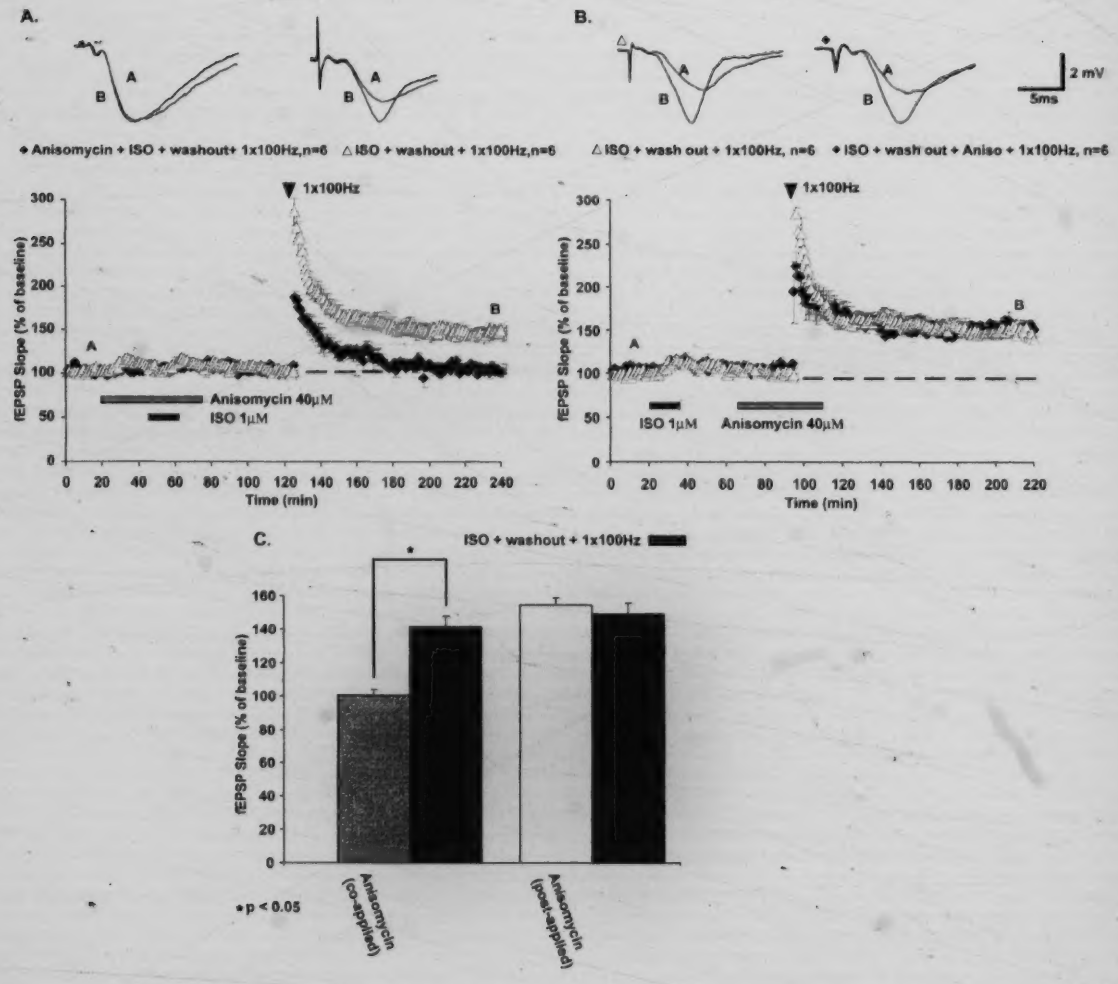

Figure 3.6. The presence of anisomycin, a translation inhibitor, in the bath during, but not after, β-AR activation blocks this form of metaplasticity. *A*, Slices treated with anisomycin during β-AR activation (black diamonds) blocked the persistence of L-LTP compared to controls (open triangles). *B*, HFS applied in the presence of anisomycin after prior β-AR activation did not impair the expression of metaplasticity (black diamonds). *C*, Comparisons of fEPSP slopes of these experiments are shown in this summary histogram. Measurements were taken 120 min after HFS application. Sample traces were taken 10min after commencement of baseline recordings and 120min after HFS protocol. Results in C represent means ± SEM (n=6).

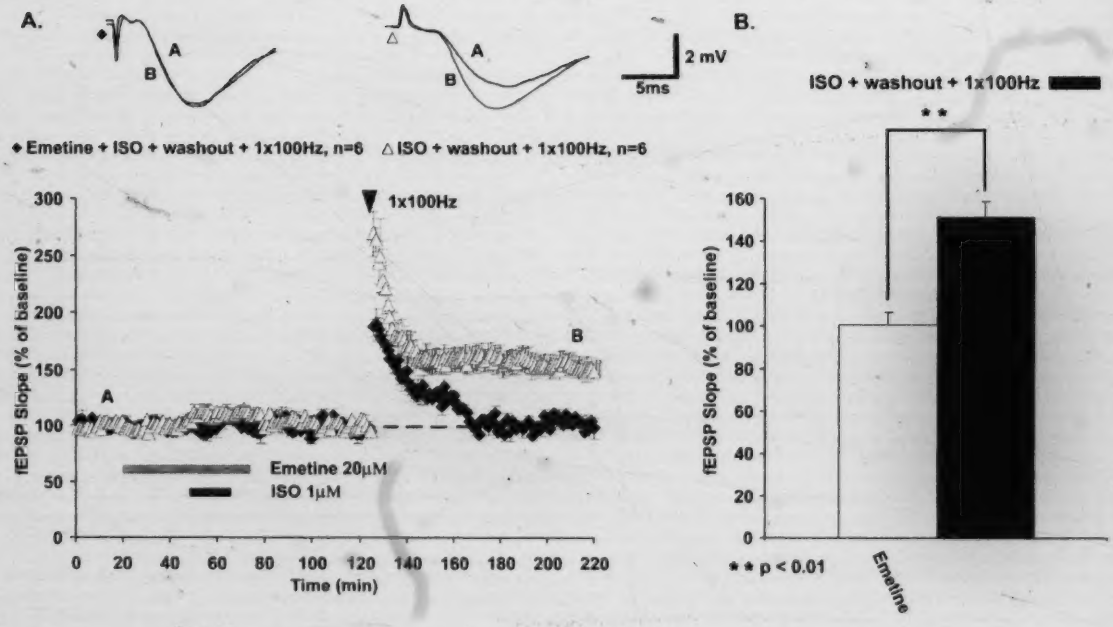

Figure 3.7. L-LTP obtained by this form of metaplasticity requires protein synthesis. *A*, Presence of emetine in the bath during the activation of the β-ARs impaired metaplasticity (black diamonds). *B*, Summary histogram comparing fEPSP slopes 120 min after HFS. Sample traces were taken 10min after commencement of baseline recordings and 120min after HFS stimulation. Results in B represent means ± SEM (n=6).

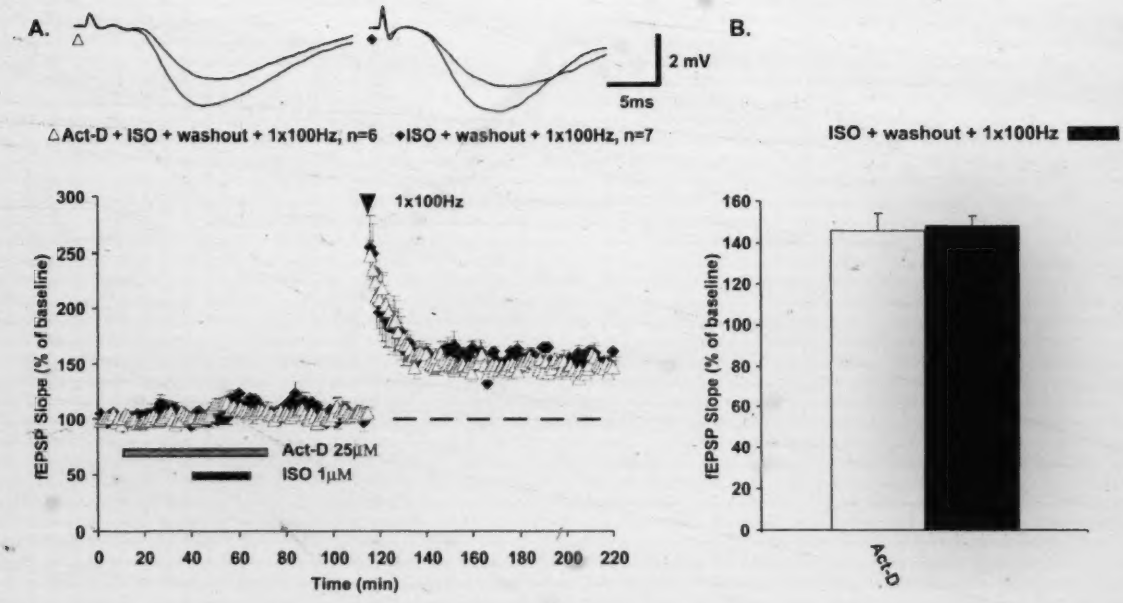

Figure 3.8. Transcription is not necessary for this β-AR-dependent metaplasticity. *A*, The presence of this transcription inhibitor did not impair metaplasticity (open triangles). *B*, Summary histogram for these experiments comparing fEPSP slopes 120 min after HFS. Sample traces were taken 10 min after commencement of baseline recordings and 120 min after HFS stimulation. Results in B represent means ± SEM (n=6).

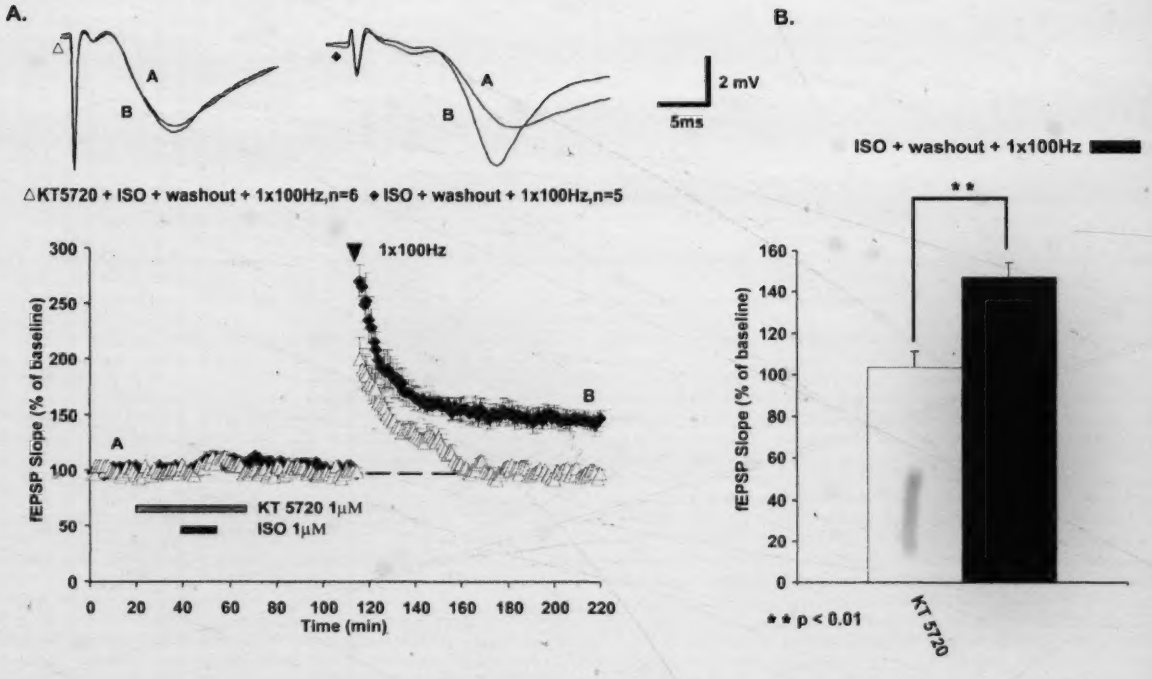

Figure 3.9. Activation of PKA is necessary for metaplasticity. *A*, 1x100Hz HFS paired with *β*-AR activation induces L-LTP (black diamonds) whereas co-application of KT5720 (a PKA inhibitor) with ISO + 1x100Hz HFS impairs L-LTP (open triangles). *B*, Summary histogram for these experiments. Sample traces were taken 10min after commencement of baseline recordings and 120min after HFS stimulation. Results in B represent means ± SEM (n=6).

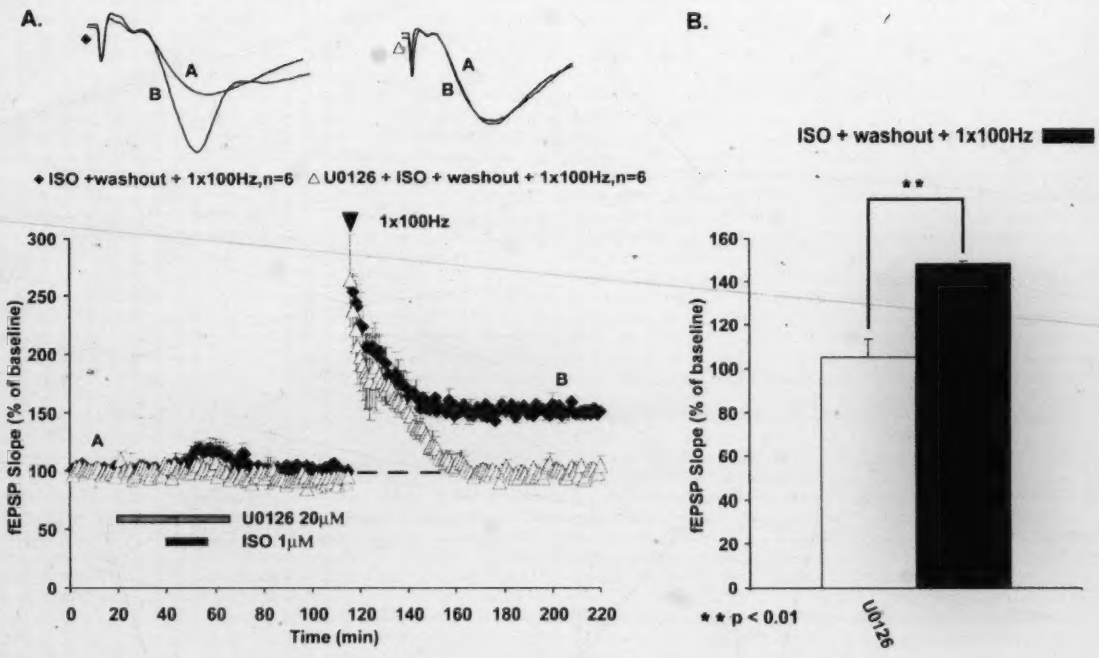

Figure 3.10. ERK is required for metaplasticity. *A*, U0126, a MEK inhibitor, was applied during β-AR activation and blocked metaplasticity (open triangles). Summary histogram comparing fEPSP slopes 120 min after HFS. Sample traces were taken 10min after commencement of baseline recordings and 120min after HFS stimulation. Results in B represent means ± SEM (n=7).

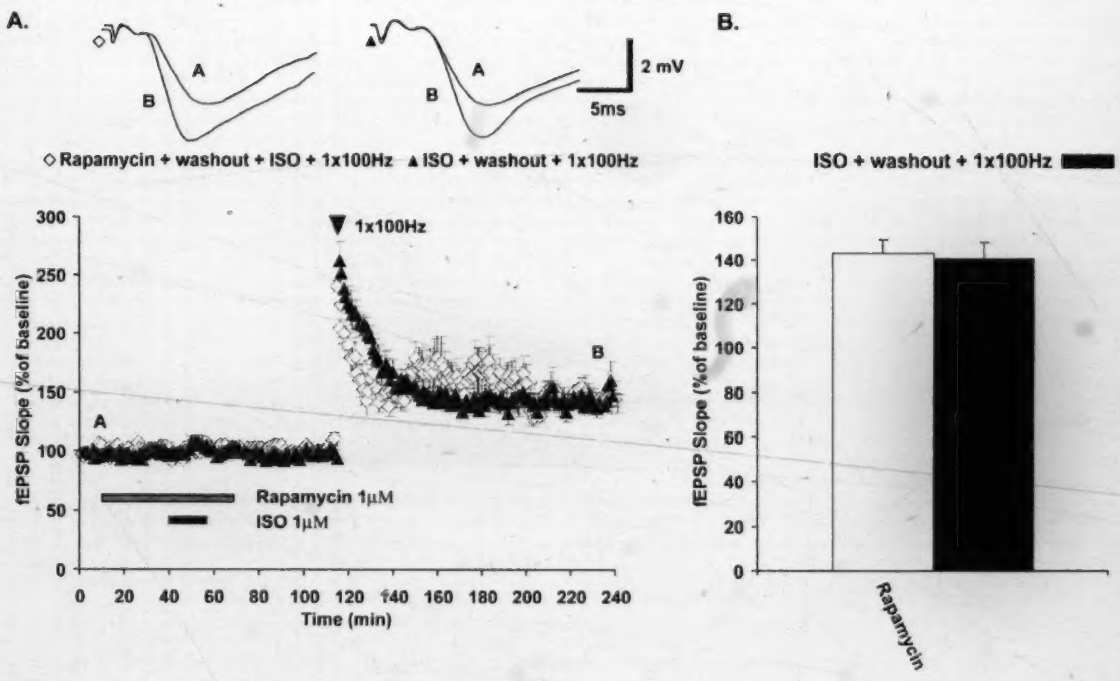

Figure 3.11. The kinase mTOR is not required for metaplasticity. *A*, When rapamycin was co-applied with ISO, fEPSPs remained potentiated (open triangles). *B*, Histogram comparing fEPSP slopes 120min after HFS. Sample traces were taken 10min after commencement of baseline recordings and 120min after HFS stimulation. Results in B represent means ± SEM (n=6).

Chapter 4 Discussion

Previous research has investigated the role of β-ARs in synaptic plasticity and how the activation of these receptors plays an important role in altering the efficacy of synapses (Thomas et al., 1996; Katsuki et al., 1997; Gelinas and Nguyen, 2005; Gelinas et al., 2007). Neuromodulators such as NA critically regulate activity-dependent synaptic plasticity in the hippocampus by altering the sensitivity of LTP to electrical stimulation. Gelinas et al. (2007) have shown that β-AR activation increases the maintenance of L-LTP when paired with 1x100Hz HFS. ISO application alone transiently increases synaptic efficacy (Gelinas and Nguyen, 2005). Previous research by Huang and Kandel (1994) has shown that 1x100Hz HFS alone induces a temporary increase in synaptic efficacy which decays back to baseline levels within 2h. However, once 1x100Hz HFS is paired with activation of β-ARs, L-LTP lasting for several hours is obtained in a protein synthesis-dependent manner (Gelinas et al., 2007).

Consistent with this idea, my results show that activation of β-ARs is required for metaplasticity since block of these receptors by propranolol prior to HFS completely abolished metaplasticity. However, block of β-ARs during HFS had no effect. These results suggest that transient activation of β-ARs is able to prime signaling pathways responsible for metaplasticity.

Gelinas et al. (2005) have shown that facilitation of L-LTP expression by β-AR activation can occur after distinct electrical stimulation protocols, as L-LTP was obtained by pairing β-AR activation not only with 1x100Hz HFS but also with 5Hz3min LFS.

Both stimulus protocols alone transiently enhance fEPSP efficiency. Thomas et al. (1996) have also demonstrated that β-AR activation potently increases synaptic strength in hippocampal area CA1 when paired with LFS. Consistent with these findings, my results show that prior activation of β-ARs reduces the threshold for future L-LTP induction by HFS. My results also suggest that activation of β-ARs extends the time frame for future induction of LTP to at least 1 h. Within this period of time, if the stimulation necessary to induce LTP is delivered, metaplasticity will be achieved. In my experiments, application of HFS 1 h after ISO activation of β-AR was able to induce L-LTP that lasted > 6 h. However, my results suggest that the time frame extension to elicit L-LTP by subthreshold stimulation is no longer than 2 h. Thus, β-AR activation initiates molecular events that apparently prime synapses to increase the time window for effective expression of future L-LTP.

NMDARs play an important role for many forms of synaptic plasticity by allowing the influx of calcium ions into the postsynaptic cell which is vital for most forms of synaptic plasticity and memory formation (Bliss and Collingridge, 1993). In area CA1, activation of these receptors increases phosphorylation of eIF4E; such phosphorylation can be inhibited by application of MEK inhibitors (upstream kinase of ERK) (Banko et al., 2004). My results show that NMDAR activation is required for β-AR-dependent metaplasticity since APV (NMDAR antagonist) blocked subsequent L-LTP expression.

Translation plays an important role in some long-lasting forms of LTP (for review, see Kelleher et al., 2004). Gelinas et al. (2007) have demonstrated a critical link between β-ARs and translation regulation, occurring via ERK- and mTOR-signaling

pathways. Consistent with these findings, anisomycin blocked metaplasticity in my studies. My results demonstrate that translation is crucial for β-AR-modulated metaplasticity. However, it is noteworthy that when anisomycin was applied overlapping with HFS (40 min after prior β-AR activation) metaplasticity was still obtained. These results suggest that prior β-AR activation primes downstream kinases involved in translation regulation and in controlling the efficacy of future LTP.

It is important to consider some non-specific effects of protein synthesis inhibitors, which include: partial inhibition of DNA synthesis, apoptosis, inhibition of catecholamine synthesis, and activation of some kinases (such as p38 MAP kinase) (Alberini, 2008). Therefore, in addition to anisomycin, I also tested emetine (EME), another protein synthesis inhibitor. The results obtained were the same as seen with anisomycin: metaplasticity expression was impaired. Thus, it is likely that the effects of anisomycin on metaplasticity do not result from non-specific effects, as both inhibitors act at distinct steps in translation (Alberini, 2008).

It has been suggested that immunity to depotentiation, the activity-dependent reversal of LTP (Staubli and Lynch, 1990; Fujii et al., 1991; Huang et al., 1999), is a characteristic of protein synthesis-dependent forms of LTP (Woo and Nguyen, 2003). Consistent with previous research, my results suggest that this form of translation-dependent metaplasticity is also immune to depotentiation.

Interestingly, research has shown that pairing subthreshold electrical stimulation with activation of β-ARs elicits LTP that is transcription-independent (Gelinas and Nguyen, 2005; Gelinas et al., 2007). My results demonstrate that prior β-AR activation functions to prime future induction of L-LTP that is also independent of transcription.

It is known that PKA can be recruited by β-AR activation (Thomas et al., 1996) and by certain types of electrical stimulation (Gelinas et al., 2008). Interestingly, LTP induced by 100Hz HFS paired with β-AR activation is PKA-independent. However, PKA is involved in the phosphorylation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), thereby facilitating the trafficking and/or stabilization of these receptors in the membrane (Abraham, 2008). This may mediate the metaplasticity seen here, but further research is needed to test this idea. In my experiments, I showed that KT5720, a PKA inhibitor, blocked β-AR-dependent metaplasticity. The fact that PKA is required for metaplasticity obtained by HFS 1 h after β-AR activation, together with my protein synthesis data (Figures 3.5., 3.6., 3.7.), suggested that ERK, an important kinase downstream of PKA that links PKA to translation (Kelleher et al., 2004), may also play a role in this metaplasticity. ERK is important for many forms of synaptic plasticity (Thomas and Huganir, 2004), including *β-LTP* (Gelinas et al., 2008). When ERK is phosphorylated, it activates MnK1 (MAPK-interacting kinase) which in turn phosphorylates eIF4E (reviewed by Kelleher et al., 2004). This phosphorylation increases translation.

In order to examine the role of ERK in metaplasticity, I applied U0126, a MEK inhibitor. I observed that inhibition of MEK impaired metaplasticity. Thus, ERK is crucial for metaplasticity. Taken together, my results suggest that PKA-ERK signaling is required for metaplasticity when HFS is applied 1 h after β-AR activation.

As shown by Gelinas et al. (2007), mTOR is an extremely important kinase in the maintenance of β-adrenergic LTP. Once activated, mTOR phosphorylates 4E-BP (eIF4E-binding protein), a repressor of eIF4E, which releases eIF4E to form the eIF4F complex,

allowing translation to commence. Therefore, mTOR was another target of my investigation, considering that this form of metaplasticity also requires translation. After applying rapamycin, an mTOR inhibitor, I observed that this inhibitor did not affect the induction or maintenance of this form of metaplasticity, suggesting that mTOR is not involved in this process.

Taken together, my results suggest that the PKA-ERK signaling pathway is crucial for this form of metaplasticity. However, mTOR (suggested as the main kinase involved in β-LTP: Gelinas et al., 2007) does not play a critical role in this novel form of metaplasticity. Thus, translation regulatory factors such as eIF4E and 4E-BP, which have been cited as factors responsible for the integration of multiple signaling pathways, may not be critical for metaplasticity. Other translational regulators may be involved. Further research is needed to test these ideas.

This fact reinforces the possibility that PKA is the key kinase involved in this process because it activates a pathway linked to translation (PKA-ERK signaling pathway), shown here to be vital for metaplasticity. In addition, PKA phosphorylates a specific site on AMPARs, serine-845 (Man et al., 2007). This phosphorylation could be the reason for the extension of the time window for priming of metaplasticity to at least 1 h seen when β-ARs are briefly activated by ISO. The proteins that are synthesized are unknown; however, it is possible that these PRPs (plasticity-related proteins) are involved in the trafficking and/or stabilization of AMPARs in the membrane.

Overall, my results extend current knowledge by showing that β-AR activation can prime synapses to enhance future LTP expression, without affecting basal transmission initially. Investigations of metaplasticity may shed light on how neurons

control levels of synaptic plasticity to prevent saturation of synaptic strength (or conversely, over-repression of synaptic strength) and, in this manner, protect against damage elicited by neural excitotoxicity or epilepsy.

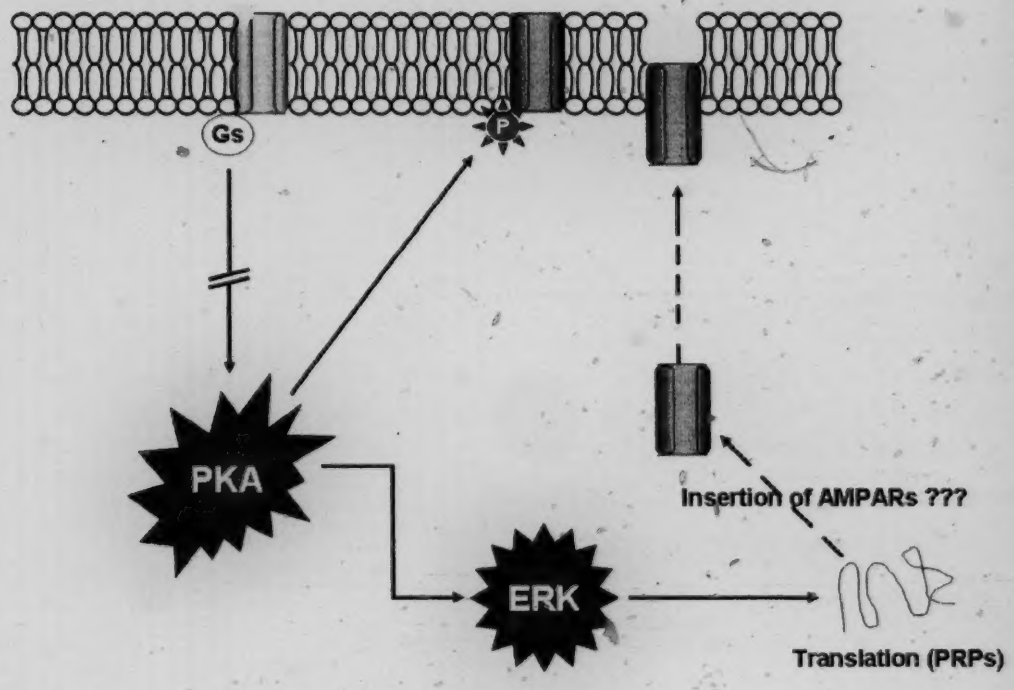

Figure 4.1. Model of β-AR-dependent metaplasticity. Activation of β-AR leads to a state of 'readiness' to downstream targets of PKA. ERK phosphorylates eIF4E through MnK1, increasing translation. PKA also phosphorylates AMPARs, which could be one of the mechanisms underlying metaplasticity in the hippocampus.

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